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Full Length Research

Spectrophotometric Determination of Phosphate Concentration in Tap Water Samples of University of Nigeria, Nsukka Community Using Molybdenum Blue Method

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Phosphate dosing of water supplies in public water system, coupled with leakage from the distribution networks represents a significant input of phosphorus to the environment. This gradual pile up of phosphate levels could directly or indirectly impact some negative environmental challenges including reduced aesthetic and recreational value of lakes, river and stream and also the seasonal depletion of the dissolved oxygen content in water bodies, thus leading to disruption of the ecosystem. The phosphate concentration in tap water samples collected from various sites in University of Nigeria, Nsukka Community was analyzed by molybdenum blue method using a simple analytical and UV-visible spectrophotometric method. The phosphate level in the water samples was measured at 840 nm. The calibration curve was developed using concentrations of 0.5-2.5 mg/L and the phosphate concentrations were obtained by calculating with the linear equation, $y = 0.172x - 0.149$, $R^2 = 0.990$. The water samples analyzed from all sampling sites showed high level of phosphates above the World Health Organization level (0.03 mg/L) with a mean concentration of 1.860 ± 0.194. Based on the findings of the study, tap water samples from various sites of University of Nigeria, Nsukka Community showed high levels of phosphates which could be due to some natural and human factors.

Keywords: Tap water, phosphate, molybdenum blue, UV-visible, spectrophotometer.

INTRODUCTION

Phosphorus and phosphate compounds are an essential nutrient necessary for the life and growth of plants and animals, because they are an important component of DNA, RNA, ATP, and phospholipids

(Sponer et al., 2012). Phosphorus commonly found in nature as phosphates is eleventh most abundant element on the surface of the earth, and was isolated from human urine and bone ash sources. This explains why phosphate mines contain fossilized deposits of animal remains and excreta (Fillippelli, 2011). The majority of phosphorus compounds are used as fertilizers. Other applications include organophosphorus compounds in detergents, pesticides, insecticides etc.

Phosphorus plays many biological roles in living cells. Phosphorus in the form of phosphate (PO_4^3) is necessary for all form of life (Dick et al., 2011), because is plays a crucial role in the structural framework of nucleic acids (DNA and RNA). Cellular energy with adenosine triphosphate (ATP) that is necessary for all cellular process that utilizes energy is transported with the aid of phosphate. ATP is also essential for phosphorylation, which is a major regulatory step in living cells (Lapel et al., 2017). Phospholipids are the major structural components of every cellular membrane of living cells. Every living cell is separated from its surrounding by the membrane that encased it. These membranes consist of a phospholipid matrix and proteins, generally in a bilayer form. Amorphous form of calcium phosphate is a component of the bone as well as hydroxyapatite.

Phosphate deficiency syndrome is characterized by hypophosphatemia, which is a medical condition of low level of soluble phosphate in the blood serum and cells. Its symptoms include neurological disorder and disruption of muscle and blood cells mainly due to lack of ATP (O'Brien and Coberly, 2003). Therefore, in 1997, the U.S Institute of Medicine (IOM) updated the Estimated Average Requirements (EARs) and Recommended Dietary Allowances (RDAs) for phosphorus, which allows daily consumption of 580 and 700 mg/day for individuals ages 19 and above (Institute of Medicine, 1997). The main sources of food containing phosphorus are the same with those containing proteins and lipids. Examples of such food include milk, meat and soya. However, an excess of phosphate can lead to complications such as diarrhea and calcification of organs and soft tissues (Moroe, 2008), which can in turn disrupt the body's ability to utilize other microminerals like calcium, magnesium, and zinc. Although organic compounds of phosphorus form a wide range of minerals, of which most are required for life; some are also severely toxic as well. For instance, chronic ingestion of white phosphorus poisoning leads to 'phossy jaw', which is necrosis of the jaw (Kamboj, 2007; Donoghue, 2005). Ingestion of white phosphorus causes severe liver damage and can cause a medical condition called 'smoking stool syndrome' (Gonzalez-Andrade and Lopez-Pulles, 2011). Fluorophosphate esters are one of the most extreme neurotoxins. Many organophosphorus compounds are used for their toxicity as pesticides (herbicides, fungicides, insecticides etc).

Public water systems usually add certain phosphates to drinking water during water distribution as a corrosive inhibitor to prevent the leaching of lead and copper from old water pipes. Lead is a toxic metal and adding phosphate has proven to be effective in reducing human exposure to lead (Watt et al., 2000). The health effects of these water treating phosphates in drinking water are not clearly known. However, the Food and Drug Administration (FDA) issued a report on these phosphate water additives to be generally considered safe (Weiner et al., 2001). Latest statistics show that water utilities in Nigeria lose about 20% of drinking water to leakage (Balogun et al., 2017; Hassan et al., 2016). Based on this estimate of phosphorus added to drinking water, this amounts to very large amount deposited into the environment including soil and rivers yearly. In the endeavor to minimize the health and environmental hazards of phosphate contamination, analysis of phosphate in water samples is necessary.

Therefore, the present study examined the amount of phosphate present in tap water samples of University of Nigeria Nsukka (UNN) Community using the molybdenum blue method.

MATERIALS AND METHODS

Chemicals

Ammonium molybdate, sulphuric acid, hydrazine hydrate, potassium dihydrogenphospate and distilled water were obtained from Department of Pure and Industrial Chemistry, UNN. Solvents were redistilled before use while reagents were used without further purification. All chemicals and reagents were of analytical reagent grade.

Instruments

The absorbance measurements were recorded using an ultraviolet-visible spectrophotometer, Jenway

Table 1. Water sample collection sites of University of Nigeria Nsukka community.

Sample label	Collection sites
W1	Girls hostels
W2	Franco avenue
W3	Odim avenue
W4	St. Peters Chaplaincy avenue
W5	Lion water production center
W6	Green house and Energy center
W7	Fulton and Margaret Catherite avenue
W8	Chitis Restaurant avenue
W9	Danfodio avenue
W10	Murtala Mohammed avenue
W11	Glen Tagart avenue

6305 model series 1800.

Preparation of phosphate standard solution

Exactly 0.717 g of potassium dihydrogenphosphate (KH_2PO_4) was carefully weighed and dissolved in distilled water and the volume was made up to 500 mL in a volumetric flask. The different concentrations of phosphate working solutions were further prepared by serial dilution of the stock solution.

Preparation of working solution of ammonium molybdate (2.5%)

50 mL of 5% of ammonium molybdate reagent was carefully measured and diluted with distilled water, and the volume made up to100 mL in a volumetric flask.

Preparation of sulphuric acid solution (10N)

28 mL of concentrated sulphuric acid solution was accurately measured and diluted with distilled water, and the volume was made up to 100 mL in a volumetric flask.

Preparation of hydrazine hydrate (0.5M)

2.44 mL of concentrated hydrazine hydrate was carefully measured and diluted with distilled water. The volume was then made up to 100 mL in a volumetric flask.

Collection of water samples (field work)

Water samples were collected from water taps from eleven different residential and commercial locations of University of Nigeria Nsukka (UNN), Enugu State, Nigeria (6º51'24"N, 7º23'45"E). The water samples used for analysis were collected from the different sites in clean plastic water bottles, in the month of August 2018. Table 1 represents a summary of the water sample collection sites of University of Nigeria, Nsukka community.

Preparation of water samples

Water samples collected from various location sites were filtered through Whatmann No. 1 filter paper to remove the insoluble particles. The filtered water samples were then stored in clean sample bottles.

Experimental method

The molybdenum blue method was used for the spectrophotometric analysis of phophorus. It is a well established analytical method for determination of inorganic phosphate. In this reaction, the acidified solutions of phosphate is treated with ammonium molybdate that gives hetero poly acid which is then reduced by hydrazine hydrate to give phosphomolybdenum blue i.e. a blue color complex [15]. At appropriate analytical conditions, the amount of phosphates in the sample solution is proportional to the intensity of the blue color.

The equation of reaction in the formation of molybdenum blue can be given as:

$$PO_4^{3-} + 12(NH_4)_2MoO_4 + 24H^+ \rightarrow (NH_4)_3PO_4.12MoO_3 + 21NH_4^+ + 12H_2O$$

$$(NH_4)_3PO4.12MoO_3 + (N_2H_4.H_2O) \rightarrow \text{molybdenum blue}$$

To determine the maximum wavelength (λ_{max}) of the phosphomolybdenum blue, the absorbance of the phosphomolybdenum blue solution was measured from 400 to 900 nm spectrophotometrically, and the wavelength at which maximum absorbance was observed was considered as the λ_{max}.

Calibration curve for the standard (KH_2PO_4)

The calibration curve is essential to establish a relationship between the absorbance and phosphate concentration for spectrophotometric analysis. Diluted solutions (1.0-5.0 mg/L) of potassium dihydrogen phosphate were prepared, and the absorbance was measured using a spectrophotometer. The absorbance readings were done in triplicates and the mean determined.

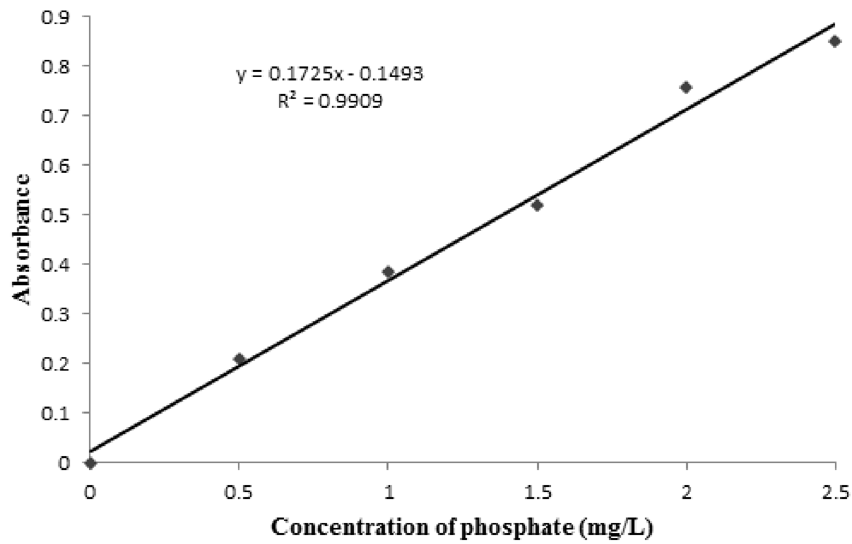

Figure 1. Calibration curve for the potassium dihydrogenphosphate standard solution.

Table 2. Absorbance of different concentration of potassium dihydrogenphosphate.

	Concentration of phosphate (mg/L)	Absorbance
1.	0.0	0.000
2.	0.5	0.210
3.	1.0	0.386
4.	1.5	0.520
5.	2.0	0.759
6.	2.5	0.851

The calibration curve is constructed by plotting absorbance values against concentration of standard solutions to give a linear equation graph.

Phosphate analysis in water samples

Phosphate analysis was carried out according to method described by Pradhan and Pokhrel (2013) with few modifications. For each case, 2 mL of the filtered water sample was accurately measured, followed by the orderly addition of 2 mL of 2.5% ammonium molybdate, 0.5 mL of 10N sulphuric acid, and 1 mL of 0.5M hydrazine hydrate solutions. The volume of the reaction mixture was made up to 25 mL in a volumetric flask by adding distilled water, and the solution was left for at least 45 min for maximum color development. Absorbance was measured using a spectrophotometer. The absorbance readings were done in triplicates and the mean determined. Then, the amount of phosphate in ppm (mg/L) was calculated from the calibration curve.

RESULTS

Determination of maximum wavelength (λ_{max})

The UV spectral data of the potassium dihydrogenphosphate used as standard against the reagent blank (water) exhibit maximum absorbance at wavelength **(λ_{max})** of 840 nm.

Calibration curve

The calibration curve for the phosphate analysis is shown in Figure 1. The curve was obtained by plotting absorbance against concentration of phosphate (mg/L) at 840 nm. The plot is linear and conforms to Beer-Lambert's law in the range of 0-2.5 mg/L (Table 2). The linear equation of the plot is y = 0.172x - 0.149, R^2 = 0.990.

Determination of phosphate in water samples

The analytical results of phosphate in water samples are represented in Table 3. The phosphate concentration in water samples was obtained by

Table 3. Absorbance and corresponding concentrations of phosphate in water samples.

Water Samples	Absorbance	Concentration of Phosphate (mg/L)
W1	0.145	1.709
W2	0.238	2.250
W3	0.179	1.907
W4	0.182	1.924
W5	0.174	1.878
W6	0.197	2.012
W7	0.132	1.634
W8	0.184	1.936
W9	0.203	2.045
W10	0.129	1.616
W11	0.157	1.779

calculating with the linear equation ($y = 0.172x - 0.149$) from the calibration curve in Figure 1. The highest concentration of phosphate was obtained from Franco Avenue (W2) with a concentration of 2.25 mg/L, and the lowest was Murtala Mohammed Avenue (W10) with a concentration of 1.616 mg/L (Figure 2).

DISCUSSION

The method employed in determination of phosphate is based on the formation of phosphomolybdate complex due to the reaction between molybdate and phosphate; the reaction is followed by its reduction with hydrazine hydrate in aqueous acidic medium. Orthophosphate and molybdate ions condense in acidic solution to give molybdophosphoric (phosphomolybdic) acid, followed by selective reduction with hydrazine hydrate to form a blue coloration. The intensity of the color is proportional to the amount of phosphate initially incorporated in the heteropolyacid (Kharat and Pagar, 2009). If the acidity at the time of reduction is 0.5 M in suphuric acid and hydrazine hydrate is the reductant, the resulting blue complex exhibits maximum absorption at 820-840 nm (Kharat and Pagar, 2009). The color intensity of the solution is measured spectrophotometrically.

The results of phosphate level in tap water samples obtained from various sampling sites in University of Nigeria Nsukka (UNN) community are presented in Table 3 and Figure 2. Findings from the study shows that all the sampling sites had phosphate level above the recommended level set by World Health Organization (WHO) for drinking and household use water i.e. 0.03 mg/L (WHO). Among the sampling sites, Franco (W2), Green house and Energy center (W6) and Danfodio (W9) had the highest level of phosphate in tap water (Figure 2). The high level of phosphate in this area is attributed to nearness of this area to farms where agricultural activities takes place all round the year. Agricultural activities are common sources of introduction of phosphate into farmlands through the application of both natural and synthetic fertilizers, and area close to these farmlands are susceptible to high phosphate level. Most of the tap water piping of the UNN community is made of asbestos, and during raining season when agricultural activities are at their peak, there may be leaching of groundwater into the asbestos pipes resulting to water enriched in phosphate ion. Another reason for high concentration of phosphate ion in water is due to industrial and human activities in the community. The dumping of refuse, laundry activities, car-washing, sewage, and human waste could also be major contributing factors. From Figure 2, it is also observed that Murtala Mohammed avenue had the lowest level of phosphates, therefore less contaminated compared to other sampling sites. The range of the phosphate levels in the sampling from highest to lowest follows; W2, W9, W6, W8, W4, W3, W5, W11, W1 and W10 respectively (Figure 2).

Phosphate dosing of drinking water supplies, coupled with leakage from distribution networks, represents a significant input of phosphorus to the environment. Gooddy et al. (2015) developed oxygen isotope composition of phosphate ($\delta^{18}O_{PO4}$), a novel

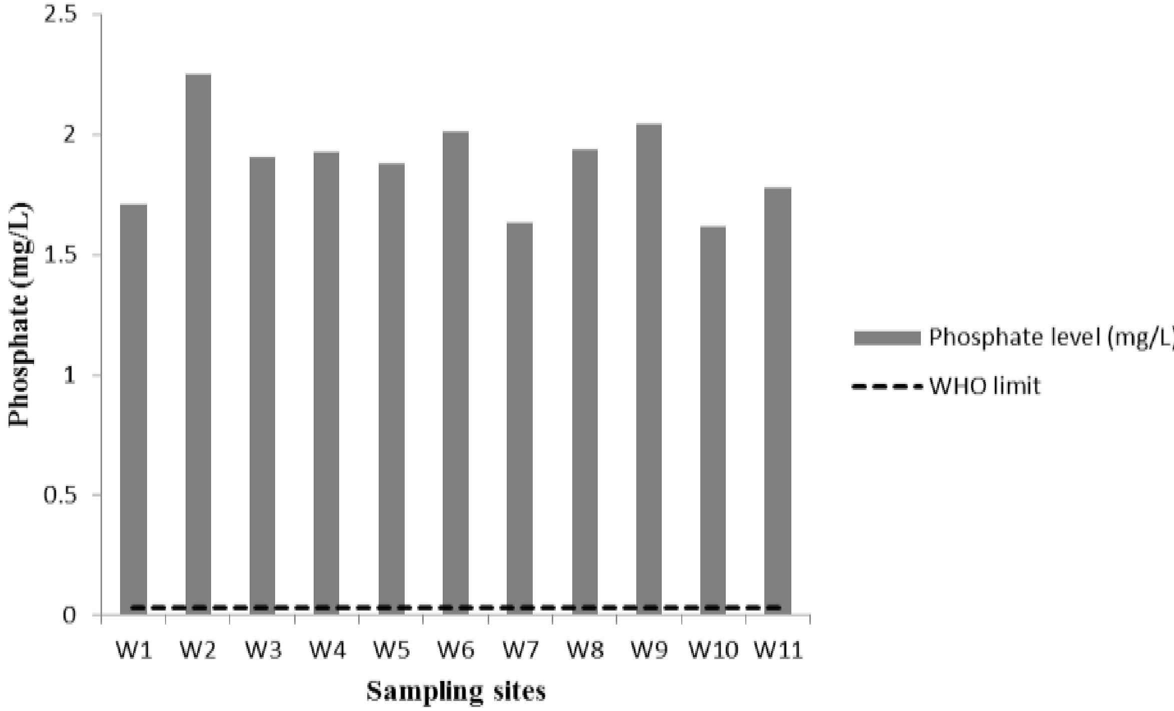

Figure 2. Phosphate level in tap water samples of University of Nigeria, Nsukka Community.

stable isotope tracer for phosphorus. This new technology offers new opportunities to understand the importance and impact of phosphorus derived from sources such as tap/drinking water. Increased phosphate levels have some negative environmental and human impacts, which includes reduced aesthetic and recreational value of lakes, river and stream and also the seasonal depletion of the oxygen content in water, thus leading to death and destruction of fish and other ecosystem (Adelowo and Oladeji, 2016).

The tap water sample analysis showed that all the samples exceeded the recommended phosphate concentration level in drinking water probably due to some natural and human factors and activities.

CONCLUSION

The spectrophotometric determination of phosphate in samples using molybdenum blue method is a simple and effective method that can be carried out in common laboratories because it does not require extraction of the analyte, or use of any sophisticated instrument such as HPLC. The results from the study showed that all sampling sites had high level of phosphate concentration in the tap water samples, and there is a possibility that leakage of phosphate ion in water from the water distribution system could lead to certain environmental and human effect on the long run. Hence, treatment and prevention methods must be considered to protect aquiferous ground water from phosphate leaching. Various treatment mechanisms such as reverse osmosis, ion-exchange etc can revive already contaminated water, while decreased use of high phosphate containing products can reduce the influx of phosphate into natural ground water.

CONFLICTS OF INTEREST

Authors declare no conflict of interests.

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